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Rapid Tween 80 Hydrolysis Test for Mycobacteria

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A rapid Tween 80 hydrolysis test for mycobacteria utilizing gas-liquid chromatography is described. The test requires 1 h of incubation followed by simple extraction and chromatography of nonderivatized oleic acid.

The conventional Tween 80 hydrolysis test and the patterns of reactivity for mycobacterial species are well documented (3). Positive reactions occur in 1 to 4 days of incubation, and 10 days of incubation are required for confirmation of negative reactions. The current study was initiated to develop a rapid procedure for the detection of Tween 80 hydrolysis by mycobacteria.

One 3-mm loopful of various mycobacterial isolates (1) was inoculated to tubes (20 by 125 mm) containing 1.2 ml of 0.5% Tween 80 (Sigma Chemical Co., St. Louis, Mo.) in 0.067 M phosphate buffer, pH 7. The tubes were incubated for 1 h at 35°C, and the nonesterified oleic acid resulting from mycobacterial esterase activity was extracted from the reaction mixture by the method of Wallach et al. (4). Briefly, the tubes were centrifuged at 2,000 × *g* for 10 min, and 1 ml of supernatant was removed, boiled for 5 min, and added to 2.5 ml of a mixture of isopropyl alcohol–heptane–1 N sulfuric acid (4:1:0.1). The samples were then mixed on a Vortex mixer; 1.5 ml of heptane was added, and the tubes were mixed as before. When the phases separated, 1.5 ml of the upper organic phase was removed and evaporated to dryness under nitrogen. The samples were redissolved in 100 μl of hexane, and 1 μl of the final concentrated extract was injected into a model 5840 gas chromatograph (Hewlett-Packard, Avondale, Pa.) equipped with a flame ionization detector. Chromatography was performed using a 6-foot (ca. 183-cm) glass (4-mm ID) Supelcoport column (Supelco, Bellefonte, Pa.) packed with 10% SP-216-PS on 100- to 120-mesh Supelcoport (2). The column temperature was 198°C; injection port and detector temperatures were both 220°C. Helium was the carrier gas at a flow rate of 80 ml/min, and the attenuation was 7. Oleic acid standard (Applied Science Laboratories, State College, Pa.) had a retention time of 16 min. Since peak separations are not a problem in the current assay, a shorter column (e.g., 2 feet [ca. 61-cm]) would reduce the retention time to approximately 5 min.

Results obtained with various mycobacterial isolates are given in Table 1. Attempts were made to transfer similar amounts of inocula for each determination. *Mycobacterium kansasii* demonstrated the greatest esterase activity, i.e., 1.3 μg of oleic acid per μl of concentrated extract. Low values of 0.1 to 0.2 μg/μl were observed with *Mycobacterium szulgai, Mycobacterium gordonae,* and *Mycobacterium terrae.* An intermediate level of activity was observed for *Mycobacterium gastri* and *Mycobacterium marinum* of 0.4 and 0.6 μg/μl, respectively. No $C_{18:1}$ peak was observed with any of the Tween 80 esterase-negative isolates. Gas-liquid chromatography results corresponded with the conventional test results with one exception. The *M. szulgai* isolate obtained from the 1976 College of American Pathologists survey (specimen E-04) was negative by the conventional procedure and positive by gas-liquid chromatography. Sixty-four percent of the survey participants had also reported the isolate negative by the conventional method.

The sensitivity of gas-liquid chromatography

TABLE 1. *Esterase activity of various mycobacterial isolates after 1 h of incubation with Tween 80*

Organism	$C_{18:1}$ peak	Conventional test
M. bovis (1)[a]	−	−
M. tuberculosis (4)	−	−
M. kansasii (5)	+	+
M. marinum (1)	+	+
M. simiae (1)	−	−
M. scrofulaceum (3)	−	−
M. gordonae (4)	+	+
M. szulgai (1)	+	−
M. intracellulare (2)	−	−
M. terrae (1)	+	+
M. gastri (1)	+	+
M. chelonei (1)	−	−

[a] The number in parentheses is number of strains tested.

and the described procedure should provide definitive data in cases where isolates give doubtful reactions with the conventional test. From these data *M. gordonae* would be recommended as a weak positive test control organism to determine the lower range of sensitivity for the test system. A negative control would detect any free oleic acid present in stock Tween 80 reagent. No free oleic acid was detected in the test reagent utilized in the current study. (Wallach et al. [4] have described a simple procedure for the extraction of free oleic acid from stock Tween 80 reagent.) For those laboratories with gas-liquid chromatography-flame ionization detection capacity, the described procedure offers a rapid method for the detection of mycobacterial esterase activity. Testing of additional isolates is needed to confirm its correlation with the conventional procedure.

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